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Amy Yost  
Amy Yost



PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Petitte *et al.*

Group Art Unit: 1632

Serial No.: 09/757,054

Examiner: Wilson, Michael C.

Filed: January 8, 2001

Docket No.: 297/93/2

Confirmation No.: 7757

For: METHOD OF PRODUCING AN UNDIFFERENTIATED AVIAN CELL CULTURE USING AVIAN PRIMORDIAL GERM CELLS

\* \* \* \* \*

DECLARATION OF JAMES N. PETITTE, PH.D.  
PURSUANT TO 37 C.F.R. §1.132

Commissioner for Patents  
P. O. Box 1450  
Alexandria, VA 22313-1450

Sir:

1. My name is James N. Petitte, Ph.D., and I am Professor of Poultry Science and the Director of the Physiology Graduate Program, at North Carolina State University, assignee for the subject U.S. Patent Application Serial No. 09/757,054.

2. A true and accurate copy of my *curriculum vitae*, which evidences my expertise and credentials, is attached herewith and labeled **Exhibit A**.

3. I have had an opportunity to review pending claims 44, 47, 48, and 51-57 in the above captioned U.S. Patent Application Serial No. 09/757,054.

4. I have also reviewed the following documents: the Official Action dated April 6, 2005 on the above captioned U.S. Patent Application Serial No. 09/757,054 by the U.S. Patent and Trademark Office (USPTO); Chang *et al.* (1995) 19 *Cell Biol Intl* 143-149 (hereinafter "Chang 1995"); Chang *et al.* (1997) 21 *Cell Biol Intl* 495-499 (hereinafter "Chang 1997"); Ponce de Leon *et al.* (1997) 21 *Revista Brasileira de Reproducao Animal* 96-101 (hereinafter "Ponce de Leon") and U.S. Patent No. 6,156,569 to Ponce del Leon et al. (hereinafter "the '569 Patent").

5. The subject matter of the pending claims relates to a sustained culture of undifferentiated avian (e.g., chicken) cells. The culture is produced by plating primordial germ cells (PGCs) isolated from the genital ridge or gonad of a post-stage 14 avian embryo on a preconditioned feeder matrix and growing the cells, which are co-isolated with stromal cells, in the presence of conditioned medium. Colonies of undifferentiated cells derived from the PGCs arise. These colonies are tightly packed and have well-defined colony borders as shown in Figure 4D of the above captioned U.S. Patent Application Serial No. 09/757,054.

6. The undifferentiated cells of the above captioned U.S. Patent Application Serial No. 09/757,054 are not PGCs, but are derived from PGCs. The undifferentiated cells have an embryonic stem (ES) cell morphology. They are smaller than the PGCs from which they are derived, and have a large nucleus, a prominent nucleolus, and relatively little cytoplasm (*i.e.*, have a high nucleus:cytoplasm ratio).

7. This morphological description of the undifferentiated cells has been used in the art for many years. For example, several patents issued to me as a co-inventor employ this descriptive terminology. U.S. Patent No. 5,340,740 states: "The established ESC lines from mouse embryos have a characteristic phenotype consisting of a large nucleus, a prominent nucleolus, and relatively little cytoplasm" (see column 1, lines 21-24). See also U.S. Patent No. 5,656,479 at column 1, lines 18-21; and U.S. Patent No. 5,830,510 at column 1, lines 22-25.

8. PGCs, in contrast to the undifferentiated cells of the claimed sustained cultures, are larger, have a lower nucleus:cytoplasm ratio, and grow individually or in loose aggregates in culture.

9. The presently claimed undifferentiated avian cells are derived from PGCs, but are not themselves PGCs or some fractional component of a population of cells isolated from the gonad or genital ridge.

10. The Patent Office's attempt to functionally define ES cells as cells that are able to contribute to the germline and somatic lineages is both over broad and too narrow. As to the former, a spermatozoan and an oocyte can give rise to all germline and somatic lineages, but one of ordinary skill in the art would not consider either to be an ES cell. With regard to the latter, there are ES cell lines available that do not give rise to germline chimeras, yet are nonetheless considered ES cells. These cell lines can still give rise to many if not all somatic lineages, and as such still can be used in the study of developmental biology and to express transgenes in these tissues.

11. Introduction of PGCs into recipient embryos results in the PGCs homing to the gonad of the embryo, where it takes part in normal gonadal development. When the embryo is born, it may be a germline chimera (dependent on the successful colonization of the gonad by the transferred PGCs), but it will not be a somatic chimera. This is shown in the '569 Patent, where all of the recipients had black feathers (*i.e.*, no PGCs contribution to the soma), but some of the offspring of the recipients had white feathers. Additionally, the Ponce de Leon journal article refers to the recipients as "putative chimeric chickens", and the authors have to breed the putative chimeras to determine whether or not they are in fact chimeric. The breeding was required because the authors were concerned with germline chimerism, which is the only chimerism that can result from the transfer of PGCs. As a result, the Patent Office's assertion on page 8 of the Official Action that "the PGCs of Ponce de Leon are ES cells because they provide

Serial No. 09/750,609

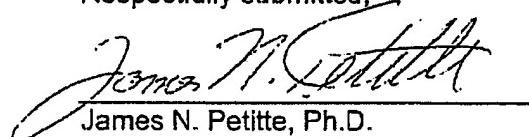
germ and somatic cell chimeras upon being introduced into recipient embryos" is not believed to be accurate.

12. PGCs do not form colonies when cultured *in vitro*. At best, PGCs in culture form loose aggregates.

13. With regard to the differences between PGCs isolated from stage 14 (Hamburger and Hamilton staging system; hereinafter "H&H") or earlier embryos versus PGCs isolated from the genital ridge or gonad of later than stage 14 embryos, prior to the instant disclosure, it was believed in the art that PGCs that had migrated to the gonad or genital ridge were committed to terminal differentiation. PGCs can be found in the genital ridge and/or gonad of later than stage 14 (H&H) chicken embryos.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

  
\_\_\_\_\_  
James N. Petitte, Ph.D.

\_\_\_\_\_  
Date

10/5/05

Attachment: Exhibit A



## CURRICULUM VITAE

James N. Petitte

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**EDUCATION**

**Ph.D., Major:** Reproductive Physiology, **Minors:** Endocrinology and Animal Breeding, 1986, University of Guelph, Guelph Ontario, Canada. (Thesis title: The influence of the adrenal gland on reproductive function and the timing of ovulation in the domestic hen.)

**M.S., Animal Sciences,** 1981, University of Maine, Orono, Maine. (Thesis title: Studies on the reproductive performance of caged broiler breeders.)

**A.B., Biology,** 1979, A.B. (magna cum laude), Susquehanna University, Selinsgrove, PA.

**PROFESSIONAL EXPERIENCE**

2001-Present	<b>Professor</b> , Department of Poultry Science, North Carolina State University, Raleigh, NC
2000-Present	<b>Director</b> , Physiology Graduate Program, College of Agriculture and Life Sciences and College of Veterinary Medicine, North Carolina State University, Raleigh, NC
1996-2000	<b>Associate Professor</b> , Department of Poultry Science, North Carolina State University, Raleigh, NC
1990-1996	<b>Assistant Professor</b> , Department of Poultry Science, North Carolina State University, Raleigh, NC
1989-1990	<b>Research Associate</b> , Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada
1989-1990	<b>Special Graduate Faculty</b> , Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada
1986-1988	<b>Postdoctoral Fellow</b> , Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada
1982-1984	<b>Graduate Teaching Assistant</b> , Department of Animal and Poultry Science, University of Guelph, Guelph, Ontario, Canada
1981-1982	<b>Instructor</b> , Department of Animal and Veterinary Sciences, University of Maine, Orono, Maine
1979-1981	<b>Graduate Teaching Assistant</b> , Department of Animal and Veterinary Sciences, University of Maine, Orono, Maine

## SCHOLARLY AND PROFESSIONAL HONORS

**Poultry Science Research Award** (1997)

**U.S.A. Branch World's Poultry Science Travel grant** (1992) to present a presentation at the World's Poultry Congress, Amsterdam.

**Canadian Branch World's Poultry Science travel grant** (1984) to present a poster at the World's Poultry Congress, Helsinki

**Taffy Davison Memorial Research Travel Grant** (1984) University of Guelph Visa Scholarship (1985) awarded to outstanding non-Canadian graduate students.

**James A. McGrath Memorial Fellowship**, Department Animal and Poultry Science, University of Guelph (1983)

**University Graduate Scholarship**, The Graduate School, University of Guelph (1983).

**University Graduate Fellowship**, The Graduate School, University of Guelph (1982).

**Hubbard Farms Scholarship**, University of Guelph (1982) and University of Maine (1979, 1980).

**Beta Beta Beta Biological Honor Society**, Susquehanna University (1978).

## PROFESSIONAL SOCIETY MEMBERSHIPS

1979 - Present	Poultry Science Association
1983 - Present	World's Poultry Science Association
1982 - Present	Society for the Study of Reproduction
1990 - Present	American Association for the Advancement of Science
1990 - Present	North Carolina Poultry Federation
1990 - Present	Triangle Reproductive Biologists
1991 - Present	Triangle Transgenic Group
1994 - 1996	Society for In Vitro Biology
1996 - Present	Society for Developmental Biology
1996 - Present	Federation of American Societies for Experimental Biology

## PUBLICATIONS (Refereed Journals)

1. Mozdziak, P.E., Q. Wu, J.M. Bradford, S. L. Pardue, C. Giamario, S. Borwornpinyo, and **J. N. Petitte**. 2005. Identification of lacZ insertion site and beta-Galactosidase expression in transgenic chickens. *Cell and Tissue Research*, in press.
2. Borwornpinyo, S., J. Brake, P. E. Mozdziak, and J. N. Petitte. 2005. Culture of chicken embryos in surrogate eggshells. *Poultry Science* 84:1477-1482.
3. Mozdziak, P.E., J. Angerman-Stewart, B. Rushton, S. L. Pardue, and **J. N. Petitte**. 2005. Isolation of chicken primordial germ cells using fluorescence-activated cell sorting. *Poul. Sci.* 84:594-600.
4. Song, Y., S. D'Costa, S. L. Pardue, and **J. N. Petitte**. 2005. Production of germline chimeric chickens following the administration of a busulfan emulsion. *Molecular Reproduction and Development*. 70:438-444.
5. Mozdziak, P. E., **J. N. Petitte**, and S. D. Carson, 2004. An introductory undergraduate course covering animal cell culture techniques. *Biochemistry and Molecular Biology Education* 32: 319-322
6. **Petitte, J. N.**, G. Liu, and Z. Yang. 2004. Avian pluripotent stem cells. *Mechanisms of Development*. 121(9): 1159-1168.
7. Mozdziak, P. E. and **J. N. Petitte**, 2004. Status of transgenic chicken models for developmental biology. *Developmental Dynamics*. 229(3): 414-421.

8. Mozdziak, P. E., S. Pophal, S. Borwornpinyo, and **J. N. Petitte**. 2003. Transgenic chickens expressing  $\beta$ -galactosidase hydrolyze lactose in the intestine. *Journal of Nutrition*. 133: 3076-3079.
9. Mozdziak, P. E., S. Borwornpinyo, D. W. McCoy, **J. N. Petitte**. 2003. Development of transgenic chickens expressing bacterial beta-galactosidase. *Development Dynamics*. 226: 439-445.
10. Giamario, C., **J. N. Petitte**, P. E. Mozdziak. 2003. Hatchability of chicken embryos following somite manipulation. *BioTechniques*. 34: 1128-1130.
11. Pardue, S.L., D'Costa, S., Song, Y and **Petitte, J.N.**, 2002. Production of inter- and intra-specific germline chimeras in poultry: potential applications. *Australian Poultry Science*. 14:54-60.
12. **Petitte, J.N.** 2002. The avian germline and strategies for the production of transgenic poultry. *Journal of Poultry Science*, 39: 205-228.
13. D'Costa, S., S.L. Pardue, and **J.N. Petitte**, 2001. Comparative Development of Avian Primordial Germ Cells and Production of Germ Line Chimeras. *Poultry and Avian Biology Reviews*. 12 (4): 151-168
14. Fasenko, G. M., V. L. Christensen, M. J. Wineland, and **J. N. Petitte**. 2001. Examining the effects of pre-storage incubation of turkey breeder egg on embryonic development and hatchability of eggs stored for four or fourteen days. *Poul. Sci.* 80 (2): 132-138.
15. Nolan, C. M., Killian, J. K., **Petitte, J. N.** and R. L. Jirtle. 2001. Imprint Status of M6P/IGF2R and IGF2 in Chickens. *Development, Genes and Evolution*. 211 (4): 179-183.
16. L. Karagenç and **J. N. Petitte**. 2000. Soluble factors and the emergence of chick primordial germ cells *in vitro*. *Poul. Sci.* 79:80-85.
17. D'Costa, S., M. J. Kulik, and **J. N. Petitte**. 2000. Expression and purification of biologically active recombinant quail stem cell factor in *E. coli*. *Cell Biology International* 24:311-317.
18. D'Costa, S. and **J. N. Petitte**. 1999. Characterization of stage-specific embryonic antigen-1 (SSEA-1) expression during early development of the turkey embryo. *International Journal of Developmental Biology* 43:349-356.
19. D'Costa, S. and **J.N. Petitte**. 1998. Rapid sex determination of turkeys using multiplex PCR. *Poultry Science* 77:718-721.
20. **Petitte, J.N.**, L. Karagenç, and M. Ginsburg. 1997. The origin of avian primordial germ cells and transgenesis in poultry. *Poultry Science* 76: 1084-1092.
21. Brake, J.T., T.J. Walsh, C.E. Benton, Jr., **J.N. Petitte**, R. Meijerhof, and G. Penalva. 1997. Egg handling and storage. *Poultry Science* 76: 144-151.
22. **Petitte, J.N.** and L. Karagenç. 1996. Growth factors during early events in avian embryo development. *Poultry and Avian Biology Reviews* 7:75-87.
23. Karagenç, L., Y. Cinnamon, M. Ginsburg, and **J. N. Petitte**. 1996. The origin of avian primordial germ cells in the prestreak embryo. *Developmental Genetics* 19:290-301.
24. **Petitte, J.N.** and M. J. Kulik. 1996. Cloning and characterization of cDNAs encoding two forms of avian stem cell factor. *Biochimica Biophysica Acta* 1307:149-151.
25. **Petitte, J.N.** and L. Karagenç. 1996. Growth factors during early events in embryo development. *Poultry Avian Biol. Rev.* 7:5-87.

26. **Petitte, J.N.**, J.M. Petitte and S. Scheideler. 1996. Determination of genetic diversity in commercial ratite stocks using multilocus DNA fingerprinting. Pages 69-77 in "Improving our understanding of ratites in a farming environment", ed. Deeming, ed., Ratite Conference: Banbury
27. Nicolas-Bolnet, C., P.A. Johnston, A.E. Kemper, C. Ricks, and **J.N. Petitte**. 1995. Synergistic action of two sources of avian growth factors on proliferative differentiation of chick embryonic hematopoietic cells. *Poultry Science* 74: 1102-1116.
28. **Petitte, J.N.** and A. E. Kegelmeyer. 1995. Rapid sex determination of chick embryos using the polymerase chain reaction. *Animal Biotechnology*, 6:119-130.
29. Yang, Z. and **J.N. Petitte**. 1994. Use of avian cytokines in mammalian embryonic stem cell culture. *Poultry Science* 73:965-974.
30. **Petitte, J.N.**, M. Kulik, and A.E. Kegelmeyer. 1994. Genomic DNA extraction from avian whole blood. *Biotechniques* 17: 664-666.
31. Qureshi, M.A., J.A. Marsh, R.R. Dietert, Y-J. Sung, C. Nicolas-Bolnet, and **J.N. Petitte**. 1994. Profiles of chicken macrophage effector functions. *Poultry Science* 73:1027-1034.
32. Millam, J.R., C.B. Craig-Veit, and **J.N. Petitte**. 1993. Brain content of cGn-RH I and II during embryonic development in chickens. *General and Comparative Endocrinology* 92: 311-317.
33. Qureshi, M.A., **J.N. Petitte**, S.M. Laster, and R.R. Dietert. 1993. Avian macrophages: contribution to cellular microenvironment and changes in effector functions following activation. *Poultry Science* 72:1280-1284.
34. Watt, J.M., **J.N. Petitte**, and R.J. Etches. 1993. Early development of the chick embryo. *Journal of Morphology* 215:165-182.
35. **Petitte, J.N.**, C.L. Brazolot, M.E. Clark, G. Liu, A.M. Verrinder Gibbins, and R.J. Etches. 1993. Accessing the genome of the chicken using germline chimeras. In: *Manipulation of the Avian Genome*, R.J. Etches and A.M.V. Gibbins, eds., CRC, Ann Arbor.
36. Havenstein, G.B., L.B. Crittenden, **J.N. Petitte**, M.A. Qureshi, and D.N. Foster. 1992. Application of biotechnology to the poultry industry. *Animal Biotechnology* 3: 15-36.
37. Brazolot, C., **J.N. Petitte**, R.J. Etches, and A.M. Verrinder Gibbins. 1991. Efficient transfection of chicken cells by lipofection and introduction of transfected blastodermal cells into the embryo. *Molecular Reproduction and Development*, 30:304-312.
38. **Petitte, J.N.**, M.E. Clark, and R.J. Etches. 1991. Assessment of functional gametes in chickens following primordial germ cell transfer. *Journal Reprod. and Fert.* 92:225-229.
39. **Petitte, J.N.** and R.J. Etches. 1991. Daily infusion of corticosterone and reproductive function in the domestic hen (*Gallus domesticus*). *General and Comparative Endocrinology* 83:397-405.
40. **Petitte, J.N.**, R.J. Etches, and C.E. Anderson Langmuir. 1991. The effect of metyrapone on the timing of oviposition and ovarian steroidogenesis in the laying hen. *British Poultry Science* 32:809-819.
41. Fasenko, G.M., F.E. Robinson, J.G. Armstrong, J.S. Church, R.T. Hardin, and **J.N. Petitte**. 1991. Variability in preincubation embryo development in domestic fowl. 1. Effects of nest holding time and method of egg storage. *Poultry Sci.* 70:1879-1881.
42. Etches, R.J. and **J.N. Petitte**. 1990. Avian and reptilian follicular hierarchies-models for ovarian development. *Journal of Experimental Zoology* S4:112-122.

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44. **Petitte, J.N.** and R.J. Etches. 1989. The effect of corticosterone on the response of the ovary to pregnant mare's serum gonadotrophin in sexually immature pullets. *General and Comparative Endocrinology* 74: 377-384.
45. **Petitte, J.N.** and R.J. Etches. 1988. The effect of corticosterone on the photoperiodic response of sexually immature fowl. *General and Comparative Endocrinology* 69: 424-430.
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47. **Petitte, J.N.**, R.J. Etches, and J.S. Walton. 1984. Normal sexual maturation and reproductive function in domestic hens following unilateral adrenalectomy. *Domestic Animal Endocrinology* 1: 189-198.
48. **Petitte, J.N.**, R.O. Hawes, and R.W. Gerry. 1983. The influence of cage vs. floor pen management of broiler breeder hens on subsequent performance of cage reared broilers. *Poultry Science* 62: 1241-1246.
49. **Petitte, J.N.**, R.O. Hawes, and R.W. Gerry. 1982. The influence of flock uniformity on the reproductive performance of broiler breeder hens housed in cages and floor pens. *Poultry Science* 61: 2166-2171.
50. **Petitte, J.N.**, R.O. Hawes, and R.W. Gerry. 1981. Control of flock uniformity of broiler breeder pullets through segregation according to body weight. *Poultry Science* 60: 2395-2400.

#### PATENTS

1. Pardue, S. L., **Petitte, J. N.**, D'Costa S and Song, Y. Methods for Gamete Production in Birds. Issued: February 17, 2004; US Patent # 6,691,638.
2. **Petitte, J. N.**, Ricks, C. A. and Spence, S. E. Gene transfer in poultry by introduction of embryo cells in ovo. Issued: February 4, 2003; US Patent # 6,515,199.
3. **Petitte, J. N.**, Ricks, C. A., Phelps, P. V. and Williams, C. Gene transfer in chickens by introduction of DNA into muscle in ovo. Issued: May 28, 2002; US Patent #6,395,961.
4. Pardue, S. L., **Petitte, J. N.**, and D'Costa, S. Methods for Gamete Production in Birds. Issued: March 12, 2002; US Patent # 6,354,242.
5. **Petitte, J. N.** and Chang, I. Method of Producing an Undifferentiated Avian Cell Culture using Avian Primordial Germ Cells. Issued: December 25, 2001; US Patent # 6,333,192.
6. **Petitte, J. N.** and Yang, Z. Veterinary Pharmaceutical Formulation Containing Avian Embryonic Stem cells. Issued: November 3, 1998; U.S. Patent # 5,830,510.
7. **Petitte, J.** and C. A. Ricks, Apparatus for Injecting Avian Embryo Muscle Tissue In Ovo, Issued: July 28, 1998, U.S. Patent #5,784,992.
8. **Petitte, J. N.** and Yang, Z. Avian Embryonic Stem Cells. Issued: August 12, 1997, U.S. Patent No. 5,656,479.

9. **Petitte, J. N.** and Yang, Z. Method of Producing an Avian Embryonic Stem Cell Culture and the Avian Embryonic Stem Cell Culture Produced by the Process. Issued: August 23, 1994, U.S. Patent No. 5,340,740.
10. **Petitte, J. N.**, Ricks, C., and Spence, S. Methods of Transferring DNA into birds by Somatic Cell Injection. U.S. Patent Pending. Filed January, 1992.
11. **Petitte, J. N.** and Ricks, C. Gene Transfer in Birds by Introduction of DNA into Muscle in Ovo. U.S. Patent Pending. Filed January, 1992.

#### **BOOK CHAPTERS**

1. **Petitte, J. N.**, 2004. Isolation and maintenance of avian ES Cells. Chapter 44, pp. 471-478 in: *Handbook of Stem Cells*, Vol. 1. *Embryonic Stem Cells*. R. Lanza, J. Gearhart, B. Hogan, D. Melton, R. Pedersen, J. Thomson and M. West, eds. Elsivier Academic Press, Burlington, MA.
2. **Petitte, J. N.** 2003. Strategies for the production of transgenic poultry. Chapter 33, pp. 665-684 in: *Poultry Genetics, Breeding and Biotechnology*. W. M. Muir and S. E. Aggrey, eds. CABI Publishing.
3. **Petitte, J. N.** and Mozdziak, P.E. 2002. Transgenic Poultry. Chapter 10 in *Transgenic Animal Technology, A laboratory Handbook*. C.A. Pinkert, Editor. Academic Press.
4. Rodriguez, G.C., D. Walmer, M. Cline, H. Kriham, R. Whitaker, P.D. Isner, B. Lessey, C. McMahon, J. Marks, **J. Petitte**, D. Carver, K. Anderson, A. Burchuck, J. Barnes, and C. Hughes, 2002. Part 4: Prevention and Screening; Chapter 23, "Biologic Effects of Progestins on Ovarian Epithelium: Cancer Prevention Through Apoptosis.", pp. 161-170, *Ovarian Cancer*, Edited by: I.J. Jacobs, J.H. Shepard, D.H. Oram, A.D. Blackett, D.M. Luesley, A. Berchuck, and C.H. Hudson, Oxford University Press
5. **Petitte, J. N.** and G. Davis. 1999. Breeding and Genetics. Chapter 11, In: *The Ostrich: Biology, Production and Health*, ed. D.C. Deeming, Wallingford, UK, CAB International.
6. **Petitte, J. N.**, S. D'Costa, and L., Karagenç. 1999. Understanding the Origin of Avian Primordial Germ Cells:Implications for Germ Cell Culture and Transgenesis in Poultry. In *Transgenic Animals in Agriculture*, eds. J.D. Murray, G.B. Anderson, A.M. Oberbauer, and M.M. McGloughlin. CAB International. P. 97-116.
7. **Petitte, J.N.**, J.M. Petitte and S. Scheideler. 1996. Determination of genetic diversity in commercial ratite stocks using multilocus DNA fingerprinting. Pages 69-77 in "Improving our understanding of ratites in a farming environment", ed. Deeming, ed., Ratite Conference: Banbury
8. **Petitte, J.N.**, C.L. Brazolot, M.E. Clark, G. Liu, A.M. Verrinder Gibbins, and R.J. Etches. 1993. Accessing the genome of the chicken using germline chimeras. In: *Manipulation of the Avian Genome*, R.J. Etches and A.M.V. Gibbins, eds., CRC, Ann Arbor.
9. Etches, R.J., J.N. Petitte, A.M. Verrinder Gibbins, C.L. Brazolot, and G. Liu. 1991. Production of chimeric chickens by blastodermal stem cell transfer and the prospects for gene manipulation. Chapter 22, pp 305-309 in: *Avian Incubation Poultry Science Symposium 22*, S.G. Tullett, ed. Butterworth-Heinemann, London.

#### **THESES SUPERVISED**

1. Song, Y. 2003. Production of mixed-sex germline chimeras in the chicken. Ph.D Thesis, NC State University.

2. Borwornpinyo, S., 2000. Optimal hatchability of cultured chicken embryos from freshly laid eggs, M.S. Thesis, NC State University.
3. D'Costa, S., 1999. Characterization of turkey primordial germ cells and the production of interspecific embryonic chimeras. Ph.D. Thesis, NC State University.
4. Karagenc, L., 1998. Development of avian primordial germ cells in vivo and in vitro. Ph.D. Thesis, NC State University.

#### **POSTDOCTORAL TRAINEES**

Zengming Yang, 1991-1992  
Carol Bolnet, 1992-1994  
Guodong Liu, 1995  
Levent Karagenc, 1999  
Il-kuk Chang, 1999  
Susan D'Costa, 2000-2001

#### **FUNDING HISTORY (Total Amount Awarded: \$2,821,649.00)**

1. NCSU Faculty Research & Professional Development Fund: Title Evaluation of Biological Changes in Transgenic Chickens. Project Leaders: C. Ashwell, P.E. Mozdziak, **J. N. Petitte**, Amount: \$15,000 Duration: 04/01/2004 - 03/31/2005.
2. Hubbard-ISA, Walpole, NH, a division of Merial. Title: A method for intra- and inter-species gamete production. Project Leaders: S. L. Pardue and **J. N. Petitte**, Amount: \$955,595.00 Duration: 1/1/2003-8/31/2005
3. Duke University (Prime--National Institutes of Health) Title: Preclinical Evaluation of Intermediate Endpoints and Their Modulation by Chemopreventive Agents : D. Carver, K.E. Anderson, **J.N. Petitte**, G. Davis, H.J. Barnes. Amount: \$379,705.00. Duration: 09/30/2000 - 08/31/2005.
4. Northwestern University (Prime--US Army-DOD) Title: Evaluation of Progestins and Vitamin D for the Chemoprevention of Ovarian Cancer. Project Leaders: K.E. Anderson, D. Carver, **J.N. Petitte**, G. Davis. Amount: \$39,639.00. Duration: 10/01/2002 - 09/30/2004.
5. Hubbard-ISA, Walpole, NH, a division of Merial. Title: A method for intra- and inter-species gamete production. Project Leaders: S. L. Pardue and **J. N. Petitte**, Amount: \$251,347. Duration: 4/15/00-12/31/02
6. Duke University: Title: Ovarian Cancer in Chickens. Project Leaders: **J.N. Petitte**, K.E. Anderson, and D. Carver, Amount: \$107,595. Duration: 10/31/99-10/31/00.
7. Duke University: Title: Preclinical Evaluation of Intermediate Endpoints and Their Modulation by Chemoprevention Agents. Project Leaders: D. K. Carver, K. E. Anderson, **J. N. Petitte**, G. S. Davis, H. J. Barnes. Amount \$399,629. Duration: 10/01/00-09/03/003.
8. Origen Therapeutics, Inc. Title: Avian Embryonic Stem Cells. Project Leader: **J.N. Petitte**, Amount: \$346,119. Duration: 7/14/97-6/30/01.
9. NSF, Title: Analysis of avian primordial germ cell development in vitro and in vivo. Project Leader, **J.N. Petitte**, Amount: \$200,000 9/15/96 - 8/31/99.

10. USDA-NRI, Title: Avian Embryonic Stem Cells, Project Leader: **J.N. Petitte**, Amount: \$165,000, Duration: 9/1/94 - 8/31/97.
11. North Carolina Biotechnology Center-ARIG, Sex-specific DNA in Ratites, Project Leader: **J.N. Petitte**, Duration: 7/1/94-12/31/96, \$39,000.
12. North Carolina Biotechnology Center-ARIG, "Establishment of Avian Blastodermal Cell Culture for the Development of Transgenic Poultry", Project Leader: **J.N. Petitte**, Duration: 8/1/91-1/31/93, \$40,000.
13. Southeastern Poultry and Egg Association "Improving Embryonic Viability of Stored Turkey Eggs. I. Effects of Preincubational Embryonic Development on Hatchability" Project Leaders: V. Christensen, M. Wineland , and **J.N. Petitte**, Amount: \$29,000 , Duration: 1/1/93-12/31/93.
14. USDA,-NRI, "Avian Embryonic Stem Cell Lines and the Development of Transgenic Poultry" Project Leader: **J.N. Petitte**, Amount: \$50,000. Duration: 9/1/91 - 8/31/93,
15. USDA/BARD, "The Study of Primordial Germ Cell Development as a Tool for Gene Transfer in chickens" Project Leader: **J.N. Petitte**, H. Eyal-Giladi and M. Ginsburg, The Hebrew University, Amount \$22,000 Duration: 10/1/92 - 9/30/95.
16. Faculty Research and Professional Development Fund, "State-Specific Antigen Expression During Development of the Early Chick Embryo", Project Leader: **J.N. Petitte**, Duration: 7/1/91-9/1/91, \$3,500.
17. North Carolina Biotechnology Center, Educational Enhancement Grant, "Faculty Training in cDNA cloning and Gene Expression", Project Leader: **J.N. Petitte**, Duration: 7/1/91-9/1/91, \$1,500.
18. Embrex, Inc., "Automated, Somatic Cell Gene Targeting Transfer in Poultry", Project Leader: **J.N. Petitte**, Amount \$152,735, Duration: 1/1/92-12/31/93.

#### **INVITED PRESENTATIONS**

1. PSA 2005 Annual Meeting, Ancillary Scientists Program, "Avian Germplasm Preservation: stem cells or PGCs?" July 30, 2005, Auburn.
2. 54<sup>th</sup> National Breeders Roundtable 2005, "Transgenic Technologies: Current Successes and Future Directions", May 5, 2005, St. Louis
3. Comparative Biomedical Sciences, College of Veterinary Medicine, "Avian Primordial Germ Cells at the Interface of Poultry Biotechnology", January 21, 2004
4. Graduate School of Bioagricultural Sciences, University of Nagoya, Japan, "Avian Embryonic Stem Cells and Transgenic Poultry", July 2001.
5. Department of Animal Science, Genetic Group Seminar, NC State University, "Avian Embryonic Stem Cells and Transgenic Poultry", April 2001.
6. USDA-ARS, Growth Biology Laboratory, Beltsville Maryland, "Avian Embryonic Chimeras and Transgenic Poultry", December 2000.
7. Department of Zoology, NC State University, "The Origin of Avian Primordial Germ Cells in the Pre-streak Embryo", March 2000.
8. Department of Poultry Science, University of Arkansas, "Biotechnology in Agriculture: Applications in the Poultry Industry", November 2000.

9. Department of Poultry Science, University of Arkansas, "Avian Embryonic Chimeras and Transgenic Poultry", November 2000.
10. World Poultry Congress, "Role of Growth Factors in Early Embryonic Development", Montreal, August 2000.
11. 12<sup>th</sup> Symposium on Current Problems in Avian Genetics, "Growth Factors in Avian Primordial Germ Cell Development", Prague, Czech Republic, September 1997.
12. Transgenic Animals in Agriculture, "Culture of Avian PGCs for Transgenesis in Poultry", Tahoe, CA, August 1997.
13. Poultry Science Association Annual Meetings, Ancillary Scientist Symposium Genetic Selection Strategies for the Future, "Primordial Germ Cells Manipulation", July 1996
14. Improving Our Understanding of Ratites in a Farming Environment, "Determination of Genetic Diversity in Commercial Ratite Stocks using Multilocus DNA Fingerprinting", Manchester England, March 1996.
15. North Carolina Emu Seminar and Trade Show, "DNA Science and the Ratite Industry: Current and Future Applications", Rockingham College, NC, November 1996.
16. 45<sup>th</sup> Annual National Breeders Roundtable, "Current Technologies for Transgenic Poultry", St. Louis, MO, May 1996.
17. Third Annual Oklahoma Ratite Seminar, "Selection of Replacement Stock", Oklahoma City, October 1995.
18. American Ostrich Breeders Association Annual Meeting, "Information Management for the Genetic Improvement of Ratites", January 1995
19. American Ostrich Breeders Association Annual Meeting, "Selection of Replacement Stock", January 1995.
20. Department of Animal Science, University of Delaware, "Avian Embryonic Stem Cells", November 1995.
21. North Carolina Ostrich Breeders Association, "Selection of Replacement Stock", November 1995.
22. American Society of Zoology, "Growth Factors in Early Embryonic Development", December 1995.
23. North Carolina Ostrich Breeders Association, "Application of DNA Science to the Ratite Industry", November 1994
24. Department of Biology, Pembroke State University, "Progress Towards Manipulation of the Avian Genome", February 1994
25. Duke Institute for Learning in Retirement, "Application of biotechnology to the Poultry Industry", February 1994.
26. Department of Molecular and Cellular Biology, Siblermen Institute of Life Sciences, The Hebrew University of Jerusalem, "Prospects for manipulation of the avian embryo using early embryonic chimeras", May 1994.
27. Animal Biotechnology Seminar Program, University of Minnesota, "Progress towards the development of transgenic poultry using avian embryonic stem cells", May 1993.
28. NCSU Biotechnology Program Retreat, NC Biotechnology Center, "Accessing the avian genome using germline chimeras", February 1993.

29. European Molecular Biology Laboratory, Heidleburg, Germany, "Development of avian embryonic chimeras and embryonic stem cells", May 1992.
30. Institute of Cellular and Molecular Embryology, CNRA College of France, Nogent sur Marne, "Progress towards the development of avian embryonic stem cells", May 1992
31. Triangle Transgenics Group, "Accessing the avian genome using blastodermal chimeras", November 1991.
32. Department of Poultry Science, University of Georgia, "Production of germ line chimeras in the chicken and the development of transgenic poultry", November 1991.
33. Department of Microbiology and Immunology, School of Medicine, East Carolina University, "Development of germline chimeras and prospects for the manipulation for the avian genome. November 1991.
34. USDA Beltsville, MD, "Development of somatic and germ line chimeras in the chicken", April 1991.
35. Keystone Symposia on Molecular and Cellular Biology: Manipulation of the Avian Genome, "Accessing the avian genome using germline chimeras", March 1991.

#### **RESEARCH ABSTRACTS**

1. Petitte, J.N., 2005. Avian germplasm preservation: stem cells or PGCs? *Poultry Sci.*, in press.
2. Pophal, S. P. E. Mozdziak, S. Borwornpinyo, and J. N. Petitte, 2004. Transgenic Chickens Expressing Beta-galactosidase Hydrolyze Lactose In The Intestine. *Poultry Sci. (Suppl. 1)* 83: 2810.
3. Giamario, C., J. N. Petitte, and P. E. Mozdziak, 2003. Hatchability of chicken embryos following intrasomite injection. *Poult Sci. (Suppl. 1)* 82: 354.
4. Tsukada, A. Kuroiwa, A., Matsuda, Y., Song, Y., Pardue, S. L., Shimada, K., and Petitte, J. N., 2003. Isolation and expression of a cDNA encoding the germ cell-specific RNA binding protein DAZL (Deleted in Azoospermia-Like) from the chicken ovary. Transgenic Animal Research Conference IV.
5. Mozdziak, P.E., S. Pophal, S., Borwornpinyo, S. Pardue, and J. N. Petitte, 2003. Transgenic chickens expressing Beta-galactosidase. Proceedings Transgenic Animals Conference. IV
6. D'Costa, S., Pardue, S.L. and Petitte, J.N. 2002. Interspecific embryonic germline chimeras produced by the transfer of gonadal PGCs. *Poultry Science*, 81(Suppl. 1):107.
7. Carver, D. K., G. Alban, H. J. Barnes, N. Reimers, B. Sanei, K. Schmitt, K. E. Anderson, and J. N. Petitte, 2002. Causes of mortality in older laying hens. *Poultry Sci. Suppl.* 81:61
8. Giamario, C., J. N. Petitte, and P. E. Mozdziak, 2003. Hatchability of chicken embryos following intrasomite injection. *Poult Sci. (Suppl. 1)* 82: 354.
9. Song, Y., D'Costa, S., Pardue, S.L., Petitte, J.N. 2002. Depletion of gonadal pgcs following the administration of a solubilized busulfan emulsion and subsequent germ cell repopulation in the chick embryo. *Poultry Science*, 81(Suppl. 1): 88.
10. D'Costa, S., Pardue, S.L., Petitte, J.N. 2002. Production of interspecific embryonic germ line chimeras by the intravascular transfer of gonadal PGCs. *Transgenic Research*, 11(1): 84-85.
11. Petitte, J.N., Z. Yang, G. Liu, M.J. Kulik and S. Borwornpinyo, 2001. Establishment of embryonic stem cells from the stage X chick embryo. 14<sup>th</sup> International Congress on Developmental Biology.

12. Borwornpinyo, S., J.T. Brake, and J.N. **Petitte**, 2001. Improved hatchability of freshly laid chicken eggs cultured *ex ovo*. Southern Poultry Science Annual Meeting
13. Borwornpinyo, S., D.W. McCoy, P.E. Mozdziak, and J.N. **Petitte**, 2001. Germline transmission of a lacZ gene in chickens using an avian spleen necrosis virus –based vector. *Poultry Science*, 80 (Suppl. 1): 42.
14. **Petitte, J. N.** 2000. Progress of the NRSP-8 Poultry Species Genome Committee. International Plant and Animal Genome Conference VIII, San Diego, CA, January 9-12, 2000.
15. Chang, I. And **J.N. Petitte**. 1999. Culture of chicken primordial germ cells on STO feeders cells. Transgenic Animal Research Conference. Lake Tahoe, 1999.
16. Petitte, J.N. K.E. Anderson, D.K. Carver, G.C. Rodreiguez, and C.L. Hughes, 1999. Apoptosis in the ovarian germinal epithelium of the domestic hen: the effect of feed restriction and levonorgestrel. Proceedings of the TCRB, January, 199.
17. Suvarna, S. V.L. Christensen, and **J.N. Petitte**. 1998. Immunohistochemical localization of the sodium-dependent glucose transporter (SGLT1) in the turkey small intestine. *Poultry Science* 77(Supp 1): 31.
18. Hunter, C.D., L. Karagenç, and **J.N. Petitte**. 1998. Expression of c-kit and SCF during the active migratory period of primordial germ cell development in the chicken. *Poultry Science* 77(Supp 1): 77.
19. D'Costa, S., and **J.N. Petitte**. 1998. Characterization of stage-specific antigen-1 (SSEA-1) as a marker for turkey primordial germ cells. *Poultry Science* 77(Supp 1): 77.
20. Anderson, K.E., **J.N. Petitte**, D.K. Carver, G.C. Rodriguez, and C. L. Huges. 1998. Effect of feed restriction and levonorgestrel on apoptosis in the ovarian germinal epithelium of the domestic hen. *Poultry Science* 77(Supp 1): 92.
21. Suvarna, S. V.L. Christensen, and **J.N. Petitte**. 1998. High levels of dietary carbohydrate increase glucose transport in turkey intestine. *Poultry Science* 77 (Supp 1): 126.
22. St. Clair, R.W., M.A. Leight, **J.N. Petitte** and J.M. Petitte. 1998. Susceptibility to atherosclerosis is reversed by bone marrow transplantation in genetically susceptible and resistant pigeons. *Circulation*, 98 (Supp): I-310.
23. **Petitte, J.N.**, L. Karagenç. 1997. Development of Avian Primordial Germ Cells (PGCs) *In vivo* and *In vitro*. 13<sup>th</sup> International Congress, Developmental Biology, 306.
24. D'Costa, S. and **J.N. Petitte**. 1997. Sex Identification of turkey embryos using a multiplex polymerase chain reaction. *Poultry Science*, 76 (Suppl. 1): 34.
25. **Petitte, J.N.**, L. Karagenç, Y. Cinnamon, and M. Ginsburg. 1997. Early germ cell development in the chick embryo. *Germ Cell Differentiation, Keystone Symposia on Molecular and Cellular Biology*, 20.
26. Suvarna, S., V.L. Christensen, W.J. Croom, and **J.N. Petitte**. 1997. Ontogeny of glucose transport in turkey intestine. *Poultry Science*, 76 (Suppl. 1): 71.
27. **Petitte, J.N.** and M.J. Kulik. 1996. Cloning of cDNAs encoding two isoforms of avian stem cell factor. *Poultry Science* 75 (Suppl.1): 94.
28. **Petitte, J.N.**, L. Karagenç, M. Ginsburg and H. Eyal-Giladi. 1996. SSEA-1 identifies the germ cell lineage in the hypoblast of stage XIII chick embryos. *Poultry Science* 75 (Suppl.1): 94.
29. **Petitte, J.N.**, L. Karagenç, J.-H. Zhou, and M. Sakurai. 1996. Growth factors and the in vitro development of chicken primordial germ cells. *Poultry Science* 75 (Suppl.1): 94.

30. Liu, G., L. Karagenç, and J. N. Petitte. 1996. Characterization of multipotent cells from early chicken embryos. *Poultry Science* 75 (Suppl. 1): 95.
31. Karagenç, L., M. Ginsburg, H. Eyal-Giladi, and J.N. Petitte. 1995. Immunohistochemical analysis of germ line segregation in preprimitive streak chick embryos using stage-specific embryonic antigen-1. *Poultry Science* 74 (Suppl. 1): 26.
32. Fasenko, G.M. V.L. Christensen, M.R. Bakst, and J.N. Petitte. 1995. Evaluating yolk membranes from short and long term stored turkey eggs using transmission electron microscopy. *Poultry Science* 74 (Suppl. 1): 44.
33. Petitte, J.N. and S.E. Scheideler. 1995. Chemiluminescent DNA fingerprinting of ratites using a PCR-labeled M13 probe. *Poultry Science* 74 (Suppl. 1): 211.
34. Fasenko, G.M., V.L. Christensen, M.J. Wineland, and J.N. Petitte. 1994. Evaluating preincubation warming of turkey eggs as a method of improving hatch ability and embryonic viability during storage. *Poultry Science* 73 (Suppl. 1): 21.
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37. Petitte, J.N. and Z. Yang, 1993. Culture of ESC-like cells from the chicken blastoderm. *Poultry Science* 72 (Suppl. 1): 95.
38. Petitte, J.N. and D. Keleman. 1992. Development of a graduate level course on biotechniques in avian biology. *Poultry Science* 71 (Suppl. 1): 157.
39. Qureshi, M.A., J.N. Petitte, S.M. Lester, and Dieter. 1992. Avian macrophages: contribution to cellular microenvironment and changes in effector functions following activation. *Poultry Science*, 71 (Suppl. 1): 315.
40. Petitte, J.N. and Z. Yang. 1992. Culture of mouse embryonic stem cells using an avian cell feeder layer or conditioned media. *Biology of Reproduction* (Suppl. 1)46: 121.
41. Petitte, J.N. and A.E. Kegelmeyer. 1992. Sex determination of chick embryos using a w chromosome specific oligonucleotide probe and PCR. Proceeding of the XIX World's Poultry Congress 1: 531.
42. Petitte, J.N., C.L. Brazolot, M.E. Clark, G.Liu, D.L. Shaw, A. M. Verrinder Gibbins, and R.J. Etches. 1991. Accessing the genome using germline chimeras in the chicken. *J. Cell. Biochem.* (Suppl.15E): 193.
43. Brazolot, C.L., J.N. Petitte, M.E. Clark, R.J. Etches, and A.M. Verrinder Gibbins. 1991. Introduction of lipofected chicken blastodermal cells into the early chicken embryo. *J. Cell. Biochem.* (Suppl. 15E): 200.
44. Watt, J.M., J.N. Petitte, and R.J. Etches. 1991. Ultrastructural morphology of the pre-primitive streak chick embryo. *J. Cell. Biochem.* (Suppl. 15E): 203.
45. Petitte, J.N. and R.J. Etches. 1990. Regional specificity of the donor cells in the development of early embryonic chimeras in the chicken. *Biology of Reproduction* (Suppl. 1) 42:176.
46. Petitte, J.N. 1989. Germ-line chimeras can be produced by embryonic stem cell transfer in the chicken.

- Cell Differentiation and Development 27: S89.
47. Brazolot, C.L., J.N. Petitte, R.J. Etches and A.M. Verrinder Gibbins. 1989. The establishment of efficient gene transfer and expression in chicken embryonic stem cells. Cell Differentiation and Development 27: S90.
  48. Petitte, J.N. and R.J. Etches. 1989. Evidence of germline chimerism after embryonic stem cell transfer. Poultry Science 68 (Suppl.1): 112.
  49. Petitte, J.N. and R.J. Etches. 1988. The development of chimeric chickens by embryonic cell transfer. Poultry Science 67 (Suppl. 1): 137.
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  51. Petitte, J.N., R.J. Etches, and J.S. Walton. 1983. Normal sexual maturation in domestic hens following unilateral adrenalectomy. Poultry Science 61:1483.
  52. Hawes, R.O. and J.N. Petitte. 1983. A comparison of the relationship of egg weight, yolk weight, and yolk sac weight with early post-hatching growth. Poultry Science 62:1433.
  53. Petitte J.N., R.O. Hawes, and R.W. Gerry. 1980. Effect of feeding different protein levels to broiler breeder pullets on flock uniformity. Poultry Science 59:1650.

## COMMITTEE ACTIVITIES

### **Departmental:**

Graduate Studies Committee  
Equipment Prioritization Committee  
Safety & Hazardous Chemicals Committee  
Seminar Committee  
Computer Committee (Chairman, 1997-2001)

### **College:**

CALS Research Committee: Chair of EPA promotions sub-committee.  
CALS Academic Computing and Advisory Committee  
CALS Safety Committee

### **Regional:**

USDA Multistate Regional Project NC-168 Genetic Improvement of Poultry  
USDA Multistate Regional Project NRSP-8 National Animal Genome Research Program

### **National:**

External Program Committee for the Transgenic Animal Conference  
Poultry Science Association:  
Ancillary Scientist Liaison Committee (Chairman)  
Poultry Science Research Award Committee  
USDA NRI: Review Panel for Competitive Grants Program  
Section: Enhancing Animal Reproductive Efficiency  
USDA IFAFS: Review Panel for Competitive Grants Program  
Section: Animal Genomics

### **Ad Hoc Grant Reviews:**

USDA National Initiative Competitive Grants Program

USDA SBIR Program  
National Science Foundation  
USDA BARD

***Ad Hoc Journal Reviews:***  
Biology of Reproduction  
Transgenic Research  
International Journal of Developmental Biology  
Poultry Science  
Journal of Veterinary Diagnostic Investigation  
Journal of Heredity  
Biotechniques  
British Poultry Science  
Journal of Experimental Zoology  
Nature Biotechnology